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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

**In re Application of:**

Havenga, *et al.*

**Serial No.:** 09/348,354

**Filed:** July 7, 1999

**For:** CHIMERIC ADENOVIRUSES

**Examiner:** Gerald G. Leffers, Ph.D.

**Group Art Unit:** 1636

**Attorney Docket No.:** 4123.2US



**DECLARATION OF MENZO HAVENGA, Ph.D.**
**UNDER 37 C.F.R. § 1.132**
**("Havenga Declaration II")**

MM
3/28/03

I, MENZO HAVENGA, Ph.D., a citizen of the Netherlands, do hereby declare and state as follows:

1. That I was awarded a degree in Biology/Biochemistry from the University of Nijmegen in 1994, and that I was awarded the degree of Doctor of Philosophy in Molecular Genetics from the University of Leiden in 1999.

2. That I am one of the joint inventors named in United States Patent Application Serial No. 09/348,354, entitled CHIMERIC ADENOVIRUSES (the "present Application"), and that the invention was the product of a collaboration between me and my co-inventors, Dr. Ronald Vogels, and Dr. Abraham Bout, both citizens of the Netherlands.

3. That I conducted (or worked directly with) the series of experiments and procedures related to this Declaration in order to generate recombinant chimeric adenoviruses based on adenovirus serotype 5 in which a part of the adenovirus serotype 5 fiber is deleted and replaced by a

part of a fiber from another adenovirus serotype.

4. That the series of experiments and procedures related to this Declaration demonstrate some of the unexpected benefits of constructing recombinant adenoviruses with chimeric fiber proteins.

5. That the enclosed Figures 1-4 do correctly summarize the discussion set forth hereinafter.

## I. Discussion

The inventors of the present Application have discovered a novel way to generate recombinant adenoviruses that have chimeric fiber proteins. (*See* Specification, pp. 32-41). One of the unexpected benefits of generating recombinant adenoviruses with chimeric fiber proteins is that the chimeric fiber proteins provide the recombinant adenoviruses with a desired antigenicity and tropism, which are different from the antigenicity and tropism of the adenovirus serotype from which the recombinant adenovirus is derived.

**FIG. 1:** As graphically depicted in FIG. 1 attached hereto, viral lysis/inhibition by serum is comparatively higher with chimeric adenoviruses, such as the Ad5.Fib16 chimera, as compared to wild-type adenovirus serotype 5. This suggests that components in the serum are responsible for the negative action towards chimeric viruses as used herein. However, this inhibiting effect towards the chimera is not due solely to neutralizing IgG's present in the serum, since a subsequent experiment shows that the IgG fraction of this serum is not sufficient to inhibit the Ad5.Fib16 chimera. FIG. 1 shows that this is the case for serum derived from Cynomolgus monkeys and from Rhesus monkeys. These experiments indicate that there are components (besides neutralizing antibodies, as suggested by Crystal et al.) in the serum that cause inhibition of the chimeric viruses.

**FIG. 2:** As graphically depicted in FIG. 2 attached hereto, chimeric adenoviruses, such as the Ad5.F35 chimera, are the subject of comparatively fewer neutralizing antibodies in Balb/c and C3H mice than is the wild-type adenovirus serotype 5 (Ad5), when injected into mice that were pre-immunized with Ad5. The solid bars (-) indicate the relative luciferase activity in untreated mice, while the open bars (+) show the relative luciferase activity (compared to 100% in non-immunized mice) in mice pre-immunized with Ad5 recombinant viruses.

**FIG. 3:** As graphically depicted in FIG. 3 attached hereto, the chimeric adenoviruses Ad5.F11, Ad5.F16, Ad5.F35 and Ad5.F50 (serotypes 11, 16, 35 and 50 are all B-group viruses) are not neutralized in C57 and Black-6 mice (Bl6) when pre-immunized with Ad5. The solid bars (-) indicate the relative luciferase activity in untreated mice, while the open bars (+) show the relative luciferase activity (compared to 100% in non-immunized mice) in mice pre-immunized with Ad5 recombinant viruses. One D-group serotype was also selected resulting in the Ad5.F13 chimera. This virus was neutralized to approximately 50% of the value found in non-immunized mice. When the non-chimera Ad5 is injected into mice that are pre-treated with Ad5, the relative luciferase activity is significantly diminished (left two bars).

**FIG. 4:** As discussed in FIG. 4 attached hereto, the chimeric adenoviruses of the present Application are expected to be substantially more stable than the adenoviruses of the Crystal patent. The chimeric adenovirus carrying the fiber of Ad7 onto the Ad5 backbone, as disclosed by Crystal and Wickham does not show a different targeting as compared to Ad5, and is presumed to bind to the same receptor (Gall et al. J. Virol. 1996 Vol. 70(4):2116-2123). The chimeric virus is not detected in lung and liver cells upon direct myocardial injection, while it is detected in lung cells upon direct lung administration. Gall et al. teaches that the receptor for Ad5 and Ad7 is the same, but that the lack of expression in the secondary sites of

infection might be due to "an inherent instability of the chimeric virus" (page 2121, 2nd column, line 11). It is believed that also neutralizing moieties such as antibodies directed against the fiber of Ad7 may play a role in the lack of activity in secondary sites, which may be solved by using a fiber from a serotype that is neutralized to a very low level, as disclosed by the present Application. The improved stability of the adenoviruses (with chimeric fibers) of the present Application is due to the retention of the tail region of the native adenovirus, which ensures proper fiber-penton base interaction. The retention of the native fiber tail region is accomplished by the method set forth at pages 32-34 of the Specification of the present Application, and the generation of chimeric adenoviruses having parts of nonnative fibers fused to the tail region of the native fiber is set forth at pages 34-41 of the Specification.

## II. Declaration

I, Menzo Havenga, do hereby declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct to the best of my information and belief.

MHavenga

Executed this 03 day of January, 2003.

Menzo Havenga, Ph.D.

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